Claim 66, see also page 76, lines 18-25 and Figures 18A and 18B. Support for Claims 67 and 68 is on page 73, lines 23-26, for example.

Rejection of Claims 40-41 Under 37 USC § 112, First Paragraph (Item 3 of Office Action)

Claims 40-41 have been rejected under 37 USC § 112, first paragraph, because "the specification, while being enabling for treating NO mediated diseases or disorders using NO donating compounds, the specification does not reasonably provide enablement for preventing diseases or disorders mediated by NO."

Claim 40 has been amended to more clearly define the invention.

Rejection of Claims 12, 15-22, 24-32, 40, 41, 42 and 45 Under 37 USC § 112, Second Paragraph (Items 4 and 5 of Office Action)

Claims 12, 15-22, 24-32, 40, 41, 42 and 45 have been rejected under 35 USC § 112, second paragraph, as being "indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention."

More specifically, in Claims 12, 15, 16 and 18, the term *low* is said to be indefinite.

The specification provides guidance to determine the meaning of the term *low* as it is used in these claims. Page 13, lines 12-25 provides a description of low molecular weight S-nitrosothiols.

Low molecular weight molecules and *small molecules* are terms of art in biology used to distinguish such molecules from *high molecular weight molecules* or *macromolecules*. See pages 6 and 7 of Lewin, B. *Genes VI*, Oxford University Press, Oxford (1997), especially Table 1.1 and text explaining it; copy provided as Exhibit AA.

Low molecular weight and high molecular weight molecules can be distinguished by the processes commonly used to purify proteins, as one of ordinary skill in the art knows. Dialysis tubing allows passage of low molecular weight molecules while retaining molecules of high molecular weight. Column chromatography based on separation by size allows the largest molecules to elute first; low molecular weight components are retarded in the commonly used size-separation chromatography media such as Sepharose. Such a test to determine which S-nitrosothiols are low molecular weight S-nitrosothiols is given on page 61, lines 17-20 of the specification. The method described is to use G-25 Sepharose (Pharmacia) column

chromatography to separate proteins from S-nitrosoglutathione, a low molecular weight S-nitrosothiol.

A further method to distinguish those molecules called *high molecular weight* and *low molecular weight* is by their precipitation properties. High molecular weight molecules are precipitable in trichloroacetic acid (commonly used at about 5% final concentration), while low molecular weight molecules remain soluble. See Exhibit 1 accompanying the Preliminary Amendment mailed to the United States Patent and Trademark Office on April 6, 1998.

See also page 56, lines 26-28, wherein "low molecular weight S-nitrosothiols" are described as being trichloroacetic acid "precipitable." In amendments to the specification made by a Preliminary Amendment mailed to the United States Patent and Trademark Office on April 6, 1998, "precipitable" has been corrected to "soluble." It should be apparent to one of ordinary skill in the art that what was meant here was that the assay was for low molecular weight thiols (high molecular weight thiols do not cross the cell membrane) and that the assay for these low molecular weight thiols involved separation from high molecular weight components by trichloroacetic acid precipitation of the high molecular weight molecules and recovery and assay of the low molecular weight molecules in a supernatant. The Examiner's attention is directed to the second and third paragraphs of the Remarks section of the Preliminary Amendment mailed to the United States Patent and Trademark Office on April 6, 1998, wherein the above-described experimental procedure is further explained as it is commonly used to differentiate between "low molecular weight" and "high molecular weight" (usually, proteins and nucleic acids) molecules, as these terms are used in the art.

Claim 16 is said to be indefinite. Claim 16 has been amended to more clearly define the invention.

Claims 16-22, 24-32 and 40-41 are said to be indefinite as to the mode of administration and the administrative amounts. Claims 16, 17, 19-22, 24, 30, 32 and 40 have been amended to add the phrase "an effective amount of" to indicate the administrative amount, as this can be determined by one of skill in the art (see page 40, line 34 to page 41, line 22. Suitable modes of administration are described on page 40, lines 26-43. Further discussion of suitable modes of administration appears on page 25, lines 15-33, page 32, lines 13-24, and on page 30, lines 24-33, for example.

Claims 21 and 22 are said to be indefinite for "a form of -*S*-NO-Hb." Claims 21 and 22 have been amended to more clearly define the invention.

Claim 40 is said to be indefinite for reciting the phrases "a disease or medical disorder which can be ameliorated ... affected by the disease or medical disorder" and "nitrosyl-heme-containing donors of NO." The term "nitrosyl-heme-containing donors of NO" is explained on page 32, lines 25-34. Claim 40 has been amended to more clearly define the invention.

In Claims 42 and 45, the NO:heme ratio is said to appear to be contradictory. Claims 42 and 45 have been amended to more clearly define the invention.

Provisional Rejection of Claims 10-16 Under 35 USC § 101 (Items 6 and 7 of Office Action)

Claims 10-16 have been provisionally rejected under 35 USC § 101 as claiming the same invention as that of Claims 9-15 of copending Application No. 08/616,371. Claims 10-16 will be canceled upon notification that Claims 9-15 of copending Application No. 08/616,371 are allowable.

Provisional Rejection of Claims 17-32 And 40-41 Under Doctrine of Obviousness-Type Double Patenting (Item 9 of Office Action)

Claims 17-32 and 40-41 have been provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over Claims 16-21 and 23-29 of copending Application No. 08/616,371. A Terminal Disclaimer is being submitted with this Amendment, thereby obviating the rejection.

Rejection of Claims 10-15 Under Doctrine of Obviousness-Type Double Patenting (Item 10 of Office Action)

Claims 10-15 have been provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over Claims 44-50 of copending Application No. 08/667,003. A Terminal Disclaimer is being submitted with this Amendment, thereby obviating the rejection.

Rejection of Claims 42 and 45 Under 35 USC § 102(b) (Item 13)

Claims 42 and 45 have been rejected under 35 USC § 102(b) as being anticipated by Moore *et al., J. Biol. Chem. 251*(9):2788-2794 (1976). The Examiner states that

> Moore et al. disclose a "stock nitrosylhemoglobin solution" which comprises nitrosylhemoglobin solution" which comprises nitrosyl-deoxyhemoglobin in a buffer and which contains an NO:heme ratio within the scope of the presently

claimed invention (e.g. greater than about 0.75) which is formed by reacting aqueous deoxyhemoglobin, buffer and NO solution. (E.g. see page 2789, left column under "Materials and Methods").

Moore *et al.* describe studies on nitrosylhemoglobin and nitrosylmyoglobin. The fourth paragraph of the Materials and Methods section of Moore *et al.* indicates that a stock nitrosylhemoglobin solution "was freshly prepared by adding stock deoxyhemoglobin solution (approximately 3 mM) to a 2-fold molar excess of 2 mM NO solution." Thus, stock solutions of deoxyhemoglobin and NO were combined to obtain a solution in which NO was at a 2-fold molar excess over hemoglobin. The NO:hemoglobin ratio of this solution was 2:1. Therefore, the NO:heme ratio was 2:4, or 0.50, since there are 4 hemes per hemoglobin molecule. In other experiments described for Fig. 6 (page 2791) of Moore *et al.*, nitrosylhemoglobin was prepared such that the NO:heme ratio was at most about 36. (Final heme concentration is stated as being 55 μM, and stock (saturated) NO solutions were 2 mM.) Dilution of NO was to 17% saturation for the lowest NO:heme ratio of about 6.

The NO:heme ratios of 0.50 and 6-36 do not fall within the range specified in Claims 42 and 45 as amended. Nowhere does Moore *et al.* propose alternative methods of preparing nitrosylhemoglobin. Nowhere in Moore *et al.* are the method of Claim 42 and the composition of Claim 45 disclosed or suggested. As amended, Claims 42 and 45 specify a ratio of NO:heme less than about 1:100, which is far different from the ratio disclosed in Moore *et al.*

Moore *et al.* do not suggest any reason why a *low* ratio of NO:heme, which would *decrease* yields of nitrosylhemoglobin, would be desirable. Moore *et al.* did not recognize the physiological application for nitrosylhemoglobin described in the specification (see discussion at page 28, line 22 to page 32, line 34, and Example 15, for instance) wherein nitrosylhemoglobin made under the conditions of Claim 42 is converted into the NO-releasing SNO-hemoglobin under physiological conditions.

Rejection of Claims 42, 45 and 46 Under 35 USC § 103(a) (Item 14)

Claims 42, 45 and 46 have been rejected under 35 USC § 103(a) as being unpatentable over Moore *et al., J. Biol. Chem. 251*(9):2788-2794 (1976) and Kharitonov *et al.*, Chapter 4, pp. 39-45 in *Methods in Nitric Oxide Research*, (Feelisch and Stamler, eds.), John Wiley and Sons, 1996. Claims 42, 45 and 46 have been amended.

As stated above in response to Item 13 of the Office Action, Moore *et al.* do not disclose or suggest the NO:heme ratios recited in Claims 42 and 45 as amended, or any NO:heme ratio close to this range. Moore *et al.* only disclose a NO:heme ratio of 0.5, and give no further guidance on how this NO:heme ratio may be varied.

Kharitonov *et al.* disclose (page 43) a method for producing nitrosylhemoglobin starting with oxyhemoglobin. Two alternative NO:heme ratios are disclosed by Kharitonov *et al.*: "stoichiometric concentrations of NO" (number 1 of page 43, column 1) and "excess NO" (paragraph spanning columns 1 and 2 of page 42). Neither of these ratios is close to the NO:hemoglobin ratio of "less than about 1:100" NO:heme ratio recited in Claims 42 and 45 as amended. Kharitonov *et al.* and Moore *et al.* do not provide any further guidance on optimizing the concentration of NO relative to that of heme to produce nitrosylhemoglobin.

The combination of the Moore *et al.* and Kharitonov *et al.* references can only suggest that the way to produce nitrosylhemoglobin is to use a NO:heme ratio of 0.5, or 6-36, NO:heme 1:1 (stoichiometric amounts) or excess NO, ratios that are all far different from the NO:heme ratios recited in Claims 42 and 45. The references do not suggest a method to produce S-nitrosohemoglobin.

There is no suggestion in either reference as to why a *low* NO:heme ratio as in Claims 42 and 45 could be desirable. Neither Moore nor Kharitonov discussed the physiological conversion of nitrosylhemoglobin to SNO-hemoglobin with the addition of oxygen.

Nothing in Moore *et al.* or Kharitonov *et al.* teaches or suggests the method of Claim 46 as amended. Neither the Moore or Kharitonov references teaches anything regarding S-nitrosohemoglobin.

Rejection of Claims 10-22, 25-28, 30-32 and 40-41 Under 35 USC § 103(a) (Item 15 of Office Action)

Claims 10-22, 25-28, 30-32, 40 and 41 have been rejected under 35 USC 103(a) as being obvious over Stamler *et al.*, WO 93/09806.

Stamler *et al.* (WO 93/09806) disclose S-nitroso-proteins, in particular, S-nitroso-tPA, S-nitroso-BSA, S-nitroso-cathepsin B, S-nitroso-lipoprotein and S-nitroso-immunoglobulin, and methods for producing the same, using NO or $NaNO_2$ as the reagent, under acidic conditions. They also report a method which they claim results in the synthesis of S-nitroso-hemoglobin. However, this compound was not produced by any method reported in WO 93/09806, as attested

to in the Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132 submitted with this Amendment. Methods used to synthesize other S-nitroso-proteins, which might have been expected to nitrosate or polynitrosate hemoglobin, dissociated hemoglobin into its subunits, oxidized the heme Fe and rendered the product useless for carrying oxygen. Methods described in the specification that result in the synthesis of nitrosated hemoglobins are substantially different from the unsuccessful acidified nitrite method described in WO 93/09806.

The Examiner states (page 9, lines 5-11):

> Stamler et al. discloses the therapeutic use of "low molecular weight" thiols, S-nitrosoprotein and amino acid compounds (e.g. S-nitroso-hemoglobin or myoglobin) for regulating protein function, inhibiting platelet function, cellular metabolism including effecting vasodilation; increasing blood oxygen transport by hemoglobin and myoglobin; NO delivery; *in vitro* nitrosylation of molecules present in the body (e.g. see Abstract; pages 1-3 and claims). Thus, the disclosed compounds are deemed useful in treating cardiovascular disorders, brain disorders and respiratory disorders within the scope of the presently claimed invention.

The claims that are the subject of the rejection are drawn to methods of treatment, methods of making NO-derivatized hemoglobins, and compositions requiring either nitrosated or nitrated hemoglobin, such as S-nitrosohemoglobin, or in the case of Claims 40 and 41, "nitrosyl-heme-containing donors of NO" such as nitrosylhemoglobin. As can be concluded from facts stated in the Declaration of Jonathan S. Stamler, S-nitrosohemoglobin was not known to exist and was not produced by the method described in WO 93/09806. "Nitrosyl-hemoglobin" is briefly mentioned on page 58, line 21 of WO 93/09806; however, nitrosylhemoglobin and other "nitrosyl-heme-containing donors of NO" are not described in WO 93/09806 as being useful for any kind of therapy. Therefore, WO 93/09806 does not and cannot teach or suggest methods for treating a medical disorder using any of these derivatives of hemoglobin or compositions comprising them.

The Examiner states (page 9, lines 11-18 of Office Action):

> Stamler discloses a thionitrosylated hemoglobin composition (e.g. see page 58 and claims 13-16) comprising reacting hemoglobin in the presence of oxygen with a nitrosating agent (e.g. SNOAc). Stamler teaches the use of equimolar amounts of nitrosating agent and Hb. Optimizing nitrosylating amounts to achieve "excess" nitrosation of hemoglobin to insure nitrosylation of hemoglobin would be obvious to the skilled artisan at the time of applicant's invention. The reference method of forming thionitrosylated oxygenated hemoglobin would render obvious the formation of thionitrosylated deoxygenated hemoglobin under anaerobic conditions as presently claimed.

WO 93/09806 teaches neither methods for making SNO-hemoglobin, as can be concluded from the Declaration of Jonathan S. Stamler, nor methods for making any other form of nitrosated hemoglobin, nitrated hemoglobin, or nitrosylhemoglobin. See item 7 of the Declaration.

The Examiner states (page 9, line 18 to page 10, line 7 of Office Action):

> The reference specifically discloses the use of nitrosylated proteins (e.g. S-nitroso hemoglobin) and low molecular weight nitrosating agents (e.g. see pages 1-2; page 24, lines 10-16) preparations thereof for the treatment of disorders by increasing oxygen capacity and transport; modulating CO and NO to tissues; scavenging radicals and vasodilation such as treating lung diseases (e.g. ARDS) and hypoxic disorders (E.g. see pages 19-25 and claims). The combination of nitrosating agents (e.g. nitrosothiol, glutathione and hemoglobin) would be prima facie obvious to the skilled artisan at the time of applicant's invention in order [to] obtain the increased pharmaceutical effects of the agents.

WO 93/09806 does not disclose the use of any species of nitrosated hemoglobin. WO 93/09806 describes the use of other *S*-nitrosylated proteins. However, S-nitrosylated hemoglobin was not produced at this time, as explained in the Declaration of Jonathan S. Stamler submitted with this Amendment. "Nitrosyl-hemoglobin" is mentioned on page 58, line 21 of WO 93/09806, but it is not described as an agent useful in any method of therapy. Nitrosylhemoglobin itself has no NO delivery function. The subject claims are drawn to methods and compositions requiring nitrosated or nitrated hemoglobin, or "nitrosyl-heme-containing donors of NO." WO 93/9806 does not and cannot describe or suggest any such methods or compositions. SNO-hemoglobin was not described in WO 93/9806. No other species of nitrosated hemoglobin is described in WO 93/09806.

Rejection of Claims 10-22, 24-32 and 40-41 Under 35 USC § 103 (Item 16 of Office Action)

Claims 10-22, 24-32 and 40-41 have been rejected under 35 USC § 103(a) because they are said to be unpatentable over Stamler *et al.* in view of Feola *et al.*, US Patent No. 5,439,882 and Klatz *et al.*, U.S. Patent No. 5,395,314.

The teachings of Stamler *et al.* (WO 93/09806) have been described above. The Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. §1.132 being filed concurrently with this Amendment states facts that lead to the conclusion that SNO-oxyHb or SNO-deoxyHb could not have been produced by the method described in Example 19 of WO 93/09806. The description provided in WO 93/09806 cannot be used to make and use SNO-oxyHb or SNO-

deoxyHb. Nitrosyl-hemoglobin is mentioned briefly in WO 93/09806, but is not described as a therapeutic agent.

The Examiner states:

> [T]he Stamler et al. reference discloses the use of *S*-nitrosating agents (e.g. low molecular weight e.g. glutathione and hemoglobin derivatives) to treat disorders by achieving a variety of physiological effects including vasodilation; radical scavenging; NO and oxygen delivery.

This conclusion cannot be drawn from WO 93/09806. According to statements in the Declaration of Jonathan S. Stamler, WO 93/09806 does not teach any modified form of hemoglobin that could produce any kind of physiological effect. The attempted synthesis of S-nitrosohemoglobin described in WO 93/09806 failed. No nitrosated or nitrated hemoglobin or nitrosyl-heme-containing donor of NO actually synthesized is described in WO 93/09806 as having a physiological effect.

The Examiner states:

> Feola *et al.* disclose the use of "blood substitutes" to restore blood volume, transport oxygen and reduce vasoconstriction (e.g. vasodilate) by the use of hemoglobin alone or combined with glutathione as a blood substitute to treat blood disorders (e.g. sickle cell anemia) (e.g. see Abstract, examples and columns 1 and 7).

Feola *et al.* (US 5,439,882) describe cross-linked mammalian hemoglobin, a method of making the same, and a method of using the same as a blood substitute. Reduced glutathione is only used in the method of synthesis to stop the cross-linking of hemoglobin when using o-adenosine as a cross-linking agent; in this case glutathione reacts through its amine to become cross-linked to a second glutathione molecule or to become cross-linked to hemoglobin. Excess glutathione is dialyzed out, so that the cross-linked hemoglobin composition contains no free low molecular weight thiol. See column 13, lines 2-6 and lines 27-30, and column 18, lines 62-64. Feola *et al.* do not teach or suggest any form of nitrosated hemoglobin or suggest any advantage for it. Nor do Feola *et al.* teach or suggest any form of a nitrosyl-heme-containing donor of NO, such as nitrosyl-hemoglobin.

Klatz *et al.* (US 5,395,314) describe an apparatus and a method to preserve organs in a cadaver or in a brain-dead patient before the organs can be removed for transplant. The method employs a solution containing perfluorocarbons, which are to act as a blood substitute and transport oxygen in a manner similar to oxygen transport by hemoglobin. The solution may also

contain antioxidants as free radical scavengers. Klatz *et al.* do not teach or suggest any modified form of hemoglobin.

The Examiner states:

> The Stamler et al. reference provides the skilled artisan with motivation to use nitrosating agents alone or combined to treat disorders or diseases to which vasodilation and oxygen/NO transport would prove to be therapeutic. It would have been obvious to the skilled artisan at the time of applicant's invention to utilize thionitrosating agents (e.g. hemoglobin, glutathione) as blood substitutes to treat blood disorders such as sickle cell anemia since the Feola reference discloses the use of hemoglobin and thiol containing blood substitutes to treat anoxic blood disorders (e.g. sickle cell anemia as disclosed by Feola) and Stamler provides a reasonable expectation that nitrosating agents will be successful to achieve the desired effects of blood substitutes.

The claims referred to above by the Examiner are not drawn to methods for using "nitrosating agents" or "thionitrosating agents" in general, but are drawn to methods for using nitrosated or nitrated hemoglobin or nitrosyl-heme-containing donors of NO. The Stamler *et al.*, reference could not provide any expectation that any nitrosated hemoglobin, including any nitrosyl-heme-containing donor of NO, could achieve any desired result as a blood substitute, as the reference does not *describe* any nitrosated hemoglobin or any effects of a nitrosated hemoglobin. As explained above, the hemoglobin composition of Feola *et al.* contains no low molecular weight thiol, and does not suggest that thiol or nitrosothiol be used in combination with hemoglobin. Feola *et al.* and Klatz *et al.* also do not mention any form of nitrosated hemoglobin or suggest any reason to administer a nitrosated hemoglobin to an animal or human. Therefore, no combination of the cited references can render the invention obvious.

The specification describes the first successful synthesis of *S*-nitrosohemoglobin. See, for example, the specification at page 47, line 32 to page 48, line 24, and page 47, lines 1-13. See also the specification at page 75, line 29 to page 76, line 6, and page 76, line 26 to page 77, line 30, for example, wherein it is described that *S*-nitrosohemoglobin can be produced from treatment of hemoglobin with NO, at appropriate NO:heme ratios, with nitrosylhemoglobin as an intermediate.

At the time of Applicants' invention, *S*-nitrosohemoglobin, the synthesis of which may appear to be described in WO 93/09806, was, in reality, still unknown (see accompanying Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132). No form of hemoglobin was known to act as a "thionitrosating agent." None of the cited references describes any physiological effect of any form of nitrosated or nitrated hemoglobin. Thus, there could have

been no reasonable expectation that hemoglobin, if it could be nitrosated or nitrated, would have any desirable effects when administered to a mammal.

The Examiner states:

> It would have been obvious to the skilled artisan at the time of applicant's invention to utilize nitrosating agents for organ preservation since the Klatz reference provides motivation to utilize compositions such as perfluorocarbons for their ability to act as "blood substitutes" and hemoglobin oxygen transporters and Stamler teaches that nitrosating agents would be successful to achieve the desired effects of blood substitutes and also act as effective hemoglobin oxygen transporters.

Claim 23, drawn to a method for enhancing the preservation of an excised organ, has been canceled.

Rejection of Claims 40 and 41 Under 35 USC 103(a) (Item 17 of Office Action)

Claims 40 and 41 have been rejected under 35 USC 103(a) because they are said to be unpatentable over Stamler, US Patent No. 5,583,101. The Examiner states:

> Stamler discloses a method of inhibiting or relaxing skeletal muscle contraction and disease states resulting therefrom by administering nitric oxide containing compounds (e.g. see Abstract and Patent claims). Stamler clearly includes the use of nitrosylated heme containing proteins including hemoglobin and serum albumin (e.g. see col. 2, lines 7-25). Accordingly, the use of a nitrosylated heme containing protein to treat a disease or disorder within the scope of the presently claimed invention would have been obvious to the skilled artisan in view of the Stamler reference since the skilled artisan would have been motivated to select a nitrosylated heme protein for use in the Stamler method.

Stamler *et al.* (US 5,583,101) describe the use of NO and compounds that release nitric oxide or deliver or transfer nitric oxide to a site of biological activity (see column 1, lines 39-52, et seq.). Stamler further states, "suitable S-nitrosylated proteins include thiol-containing proteins ... such as ... heme proteins such as hemoglobin...." The synthesis of S-nitrosylated hemoglobin is not described in 5,583,101; there is only a reference to "PCT Published Application No. WO 93/09806, published May 27, 1993." As can be concluded from the accompanying Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132, S-nitrosylated hemoglobin was not actually produced as described in the PCT application referred to in 5,583,101 and was not known to exist before the filing of the priority application. The Stamler *et al.* patent refers specifically to S-nitrosylated proteins; no other species of nitrosated hemoglobin such as

nitrosylhemoglobin is mentioned in 5,583,101. It was not known, until described in the subject application, that the NO-delivering compound S-nitrosohemoglobin can be made by the addition of nitric oxide to hemoglobin under the appropriate conditions.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (781) 861-6240.

Respectfully submitted,

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GENES VI

Benjamin Lewin

Oxford New York Tokyo
Oxford University Press
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The diameter of the ribosome (the machine that synthesizes proteins) is comparable to that of a microtubule (a common filamentous structure that is part of the skeleton of the cell). The ribosome is large compared to an individual nucleic acid or protein. But moving to the cellular scale in **Figure 1.3**, the ribosome seems small relative to a nuclear pore (the means of communication between nucleus and cytoplasm). The nucleus of an animal cell is ~50× the size of a bacterium; and a single human chromosome may be larger than a bacterium.

Macromolecules are assembled by polymerizing small molecules

All living organisms consist of cells, and we may view the molecules of which cells are made as falling into two general classes:

- **Small molecules** are the substrates and products of metabolic pathways, providing the energy needed for cell survival. They fall into four general classes: **sugars, fatty acids, amino acids,** and **nucleotides.**

- **Polymeric molecules**—the structural components of the cell—are synthesized from the small molecules. When a small molecule is incorporated into a polymer, it is sometimes described as a **subunit** of that structure (see Table 1.2). The four types of these assembly reactions are:
  polysaccharides are assembled from sugars;
  lipids are assembled from fatty acids;
  proteins are assembled from amino acids;
  nucleic acids are assembled from nucleotides.

Each type of polymer consists of a series of subunits of the appropriate type, usually connected end to end. A biological polymer is assembled by a process in which individual subunits are added one by one via a covalent bond to the chain so far assembled. Because covalent bonds are intrinsically stable under physiological conditions, a biological polymer can survive indefinitely in a living cell in the absence of any specific attack on the bond.

Each polymerization reaction is accompanied by the loss of a molecule of water for every subunit added, giving rise to the name **condensation reaction.** To reverse the polymerization reaction by introducing a break in a chain requires the addition of a water molecule, and this therefore falls into the general class of a **hydrolytic** reaction. Reactions in which protein chains are cleaved are called **proteolytic;** those in which nucleic acids are cleaved are called **nucleolytic.**

Table 1.1 views the bacterial cell in terms of its molecular components. The major part of the mass, of course, consists of water (actually a dilute salt solution containing many inorganic ions). Proteins provide the major component of the dry mass (~15%), and the nucleic acids (DNA and RNA) altogether comprise another

Table 1.1 Considering a bacterium in terms of its molecular components. (The overall mass is ~ 7×10^{-13} g.)

Component	Proportion of Cell Mass
Inorganic	
Water	70%
Inorganic ions	1%
Organic	
Carbohydrate	3%
Amino acids	0.5%
Nucleotides	0.5%
Large molecules	
Proteins	15%
DNA	0.5%
RNA	6%
Polysaccharides	2%
Lipids	2%

appreciable component (~5%). The small molecules, comprising metabolic intermediates and the precursors to the macromolecules, together make up <5% of the mass.

Proteins and nucleic acids are *very large molecules*, known as **macromolecules.** They are responsible for conveying genetic information. Nucleic acids carry the information, while proteins provide the means of executing it. In each case, *the sequence in which the individual building blocks are joined is the critical feature that determines the property of the resulting macromolecule.*

Polysaccharides also may be large enough to qualify as macromolecules, although they do not have the complexity (in the informational sense) of proteins and nucleic acids. Lipids are not usually large enough to classify formally as macromolecules.

Biological polymers can be described in terms of a general type of organization. A polymer has:

- A **backbone** consisting of a regularly repeating series of bonds.
- **Side-groups** of characteristic diversity that stick out from the backbone.

Figures 1.4–1.6 illustrate the general structure of each type of biological polymer diagrammatically. The backbone is constructed by joining the repeating units (shaded in purple); the side-groups (shown in blue or red) are joined to the backbone. Proteins and nucleic acids are strictly linear; polysaccharides can have branches. **Table 1.2** summarizes the types of links in each backbone and the extent of diversity that can be conferred by the side-groups. Proteins are highly diverse, nucleic acids have some variety, but polysaccharides have little diversity in the types of side-groups. We now consider the structures of proteins in more detail; and in Chapter 4 we consider the properties of nucleic acids.

Figure 1.4 A protein has a simple polypeptide backbone and a wide variety of side-groups.

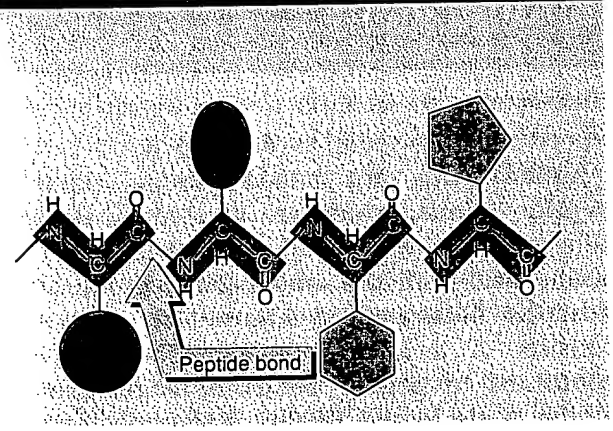

Figure 1.5 A nucleic acid has a sugar-phosphate backbone, and only four types of side-group.